Viviparity in the Sea Star *Cryptasterina hystera* (Asterinidae)—Conserved and Modified Features in Reproduction and Development

MARIA BYRNE

Department of Anatomy and Histology, F13, University of Sydney, NSW 2006, Australia

Abstract. *Cryptasterina hystera* has a highly derived life history with intragonadal development and juveniles that emerge from the parent's reproductive tract. The gonads are ovotestes with developing eggs separated from sperm by follicle cells. *C. hystera* has typical echinosperm that must enter the gonoduct of conspecifics to achieve fertilization. During oogenesis, an initial period of yolk accumulation is followed by hypertrophic lipid deposition, the major contributor to the increase in egg size. 1-Methyladenine induces egg maturation and ovulation, but the spawning component of the hormonal cascade is suppressed. This is the major alteration in reproduction associated with evolution of viviparity in *C. hystera*. The switch to viviparity was not accompanied by major change in gonad structure, indicating there were few or no anatomical constraints for evolution of a marsupial function for the gonad. Despite their intragonadal habitat, the brachiolaria are equipped for a planktonic life, swimming in gonadal fluid. During the gastrula stage, lipid provisions are released into the blastocoel where they are stored for juvenile development. The eggs of *C. hystera* have light and dark cytoplasmic regions that mark animal-vegetal polarity. The dark pigment provided a marker to follow the fate of vegetal cells. Live birth is rare in the Echinodermata and the incidence of this form of brooding in the phylum is reviewed.

Introduction

A decoupling of selective forces on the larval and adult life stages of marine invertebrates has resulted in a remarkable diversity of life-history patterns. Markedly different larval stages within closely related species often contrast with the comparative similarity of the adults. This is exemplified by cryptic morphospecies, where new species have been discovered through observation of differences in life history, reproductive anatomy, or molecular sequence data (Reid, 1990; Knowlton, 1993; Degnan and Lavin, 1995; Ó Foighil and Smith, 1995; Byrne *et al.*, 1999a, 2003a; Huber *et al.*, 2000). Within major marine groups, some taxa (*e.g.*, syllid polychaetes, littorinid snails, lasaeid bivalves, asterinid sea stars) show greater variation in life-history patterns than closely related taxa (Pocklington and Hutcheson, 1983; Reid, 1990; Ó Foighil and Smith, 1995; Byrne *et al.*, 1999a). Why this is so is a major question for phylogeny and developmental evolution.

Within the Echinodermata, the Asterinidae, a major family of sea stars, is particularly noted for its diverse life histories (Byrne and Cerra, 1996; Hart *et al.*, 1997, 2003, 2004; Byrne *et al.*, 1999a). Species in the asterinid genera *Patiriella* and *Cryptasterina* exhibit a range of developmental modes, including a most derived method of propagation—incubation of progeny in the gonads and birth of juveniles (Byrne, 1996; Byrne and Cerra, 1996; Byrne *et al.*, 2003a). In the Asterinidae, new species have been discovered by observation of developing stages in the gonads or birth of juveniles (Dartnall, 1969; Keough and Dartnall, 1978; Hart *et al.*, 2003; Byrne *et al.*, 2003a; Dartnall *et al.*, 2003). These asterinids constitute a suite of recently diverged cryptic species in which some species exhibit as little as 1% difference in mtDNA sequence compared with congeners that have dispersive larvae (Hart *et al.*, 2003; Byrne *et al.*, 2003a).

This study documents reproduction and development in the most recently discovered viviparous species, *Cryptasterina hystera*, a member of the former pan-tropical *Patiriella pseudoexigua* group (Dartnall *et al.*, 2003). Unexpectedly, *C. hystera* has a typical lecithotrophic brachiolaria

(Byrne *et al.*, 2003a). By contrast, viviparous *Patiriella* species have a vestigial brachiolaria (Byrne and Cerra, 1996). Viviparity in *C. hystera* appears to have arisen from a free-spawning ancestor with a planktonic lecithotrophic brachiolaria larva through retention and intragonadal fertilization of a large egg (Byrne *et al.*, 2003a). The gonad of *C. hystera* is an ovotestis and serves as a marsupium for the developing embryos. Reproduction and development of *C. hystera* were examined in this study to determine which life-history traits are conserved and which traits have been modified in association with the evolution of viviparity. Sperm ultrastructure was examined for modifications associated with internal fertilization. The embryos of *C. hystera* are provisioned with a conspicuous store of lipid (Byrne *et al.*, 2003a). Recent studies indicate that maternal lipid reserves are used to support juvenile development (Emlet and Hoegh-Guldberg, 1997; Byrne and Cerra, 2000; Villinski *et al.*, 2002; Byrne *et al.*, 2003b), and particular attention was paid to the fate of these reserves during development of *C. hystera*. The eggs of *C. hystera* have light and dark cytoplasmic regions that mark animal-vegetal polarity. The dark pigment was used as a marker to follow the fate of vegetal cells in development of larval territories.

Materials and Methods

Mature specimens of *Cryptasterina hystera* were collected from Statue Bay (23°15'S; 150°45'E), Queensland, during the reproductive period September–December 1997 and 1999. Ova were obtained by placing gonad lobes that appeared to be entirely female in the ovulatory hormone 1-methyladenine (10^{-5} *M* in filtered seawater [FSW]). Eggs released from the gonad were fertilized by sperm from the same or a different specimen. The larvae were reared in FSW at 21 °C until metamorphosis.

For histology, the gonads were fixed in Bouin's fluid for 24 h, rinsed in distilled water, dehydrated in graded ethanols, and embedded in paraffin. Serial sections were stained with hematoxylin and eosin. For light and transmission electron microscopy (TEM), gonads, larvae, and juveniles were fixed for 1 h at room temperature (RT) in 2.5% glutaraldehyde in 0.45 μm FSW, and then rinsed in 2.5% NaHCO$_3$ (pH 7.2). The specimens were post-fixed in 2.0% osmium tetroxide in 1.25% NaHCO$_3$ for 1 h at RT. Other specimens were fixed in 3% glutaraldehyde in 0.2 *M* cacodylate buffer (pH 7.4) with NaCl (30 mg/ml) added to the primary fixative for 2 h at 4 °C. This was followed by four rinses in 0.2 *M* cacodylate buffer with reduced amounts of NaCl added to each rinse, with the final rinse containing no NaCl. The specimens were post-fixed in 1.0% osmium tetroxide in 0.2 *M* cacodylate buffer for 2 h at 4 °C. Tissues fixed by both methods were rinsed in distilled water, dehydrated in graded ethanols, and embedded in Spurr's resin. Semithin sections were stained with 1% toluidine blue for

light microscopy. Ultrathin sections were stained with 2% uranyl acetate for 30 min and 2.0% lead citrate for 10 min. The sections were viewed with a Phillips EM400 transmission electron microscope.

Results

Hermaphroditic gonad

The gonads of *Cryptasterina hystera* were a mosaic of oogenic and spermatogenic areas (Figs. 1A; 2B, D; 3A). As characteristic of asteroids (Chia and Koss, 1995), the gonad wall was formed by two tissue layers separated from each other by the genital coelom (Fig. 2D). The outer layer consisted of the outer peritoneum, a connective tissue layer, and the epithelial lining of the genital coelom (Fig. 2E). The inner layer also had three layers: the coelomic epithelium, a connective tissue layer, and the inner germinal epithelium (Fig. 2E). Both coelomic epithelia contained myoepithelial cells and occasional neurons. The inner connective tissue was the widest tissue layer in the gonad wall. It contained flocculent hemal fluid and occasional coelomocytes. The germinal layer contained somatic cells and developing gametes.

The eggs developed within a follicle formed by somatic cells (Fig. 2D–F). Follicle cells extended from the germinal epithelium to surround the egg, thereby separating them from adjacent sperm (Fig. 2E, F). Oogenesis in *C. hystera* was characterized by an initial period of yolk granule formation (Fig. 2A) in primary oocytes ($\bar{x} = 25$ μm diam; SE = 0.71 μm; $n = 15$), followed by lipid accumulation. The accumulation of lipid in larger oocytes, starting at a mean diameter of 48 μm (SE = 0.9 μm; $n = 21$), was responsible for the major increase in egg size (Fig. 2B, C). Yolk granules were interspersed with lipid droplets, and some granules were pushed towards the egg cortex (Fig. 2B). Fully grown eggs (440 μm diameter) were dominated by lipid. A vitelline layer and jelly coat surrounded the eggs, and cortical granules were dispersed along the oolemma (Fig. 2B, E, F). Lipid droplets were not present in the cortical cytoplasm (Fig. 2B). Although looked for, egg maturation and ovulation were not observed and ova were not found in sections.

Large eggs were distinctly marked by pale olive green and dark-colored cytoplasmic regions (Fig. 1A, B). The vegetal hemisphere (confirmed by site of gastrulation) was marked by dark pigment (Fig. 1E). A small dark spot at the animal pole marked the position of the germinal vesicle (Fig. 1A, C). When the gonads were dissected, the eggs floated to the surface, animal pole up (Fig. 1B). Ultrastructural examination of the eggs did not reveal any structures at the vegetal pole that might be connected with the accumulation of dark pigment.

Spermatogenesis was usually allocated to small regions of the germinal epithelium, although entire lobes of some gonads were devoted to sperm production (Fig. 1A). Sperm
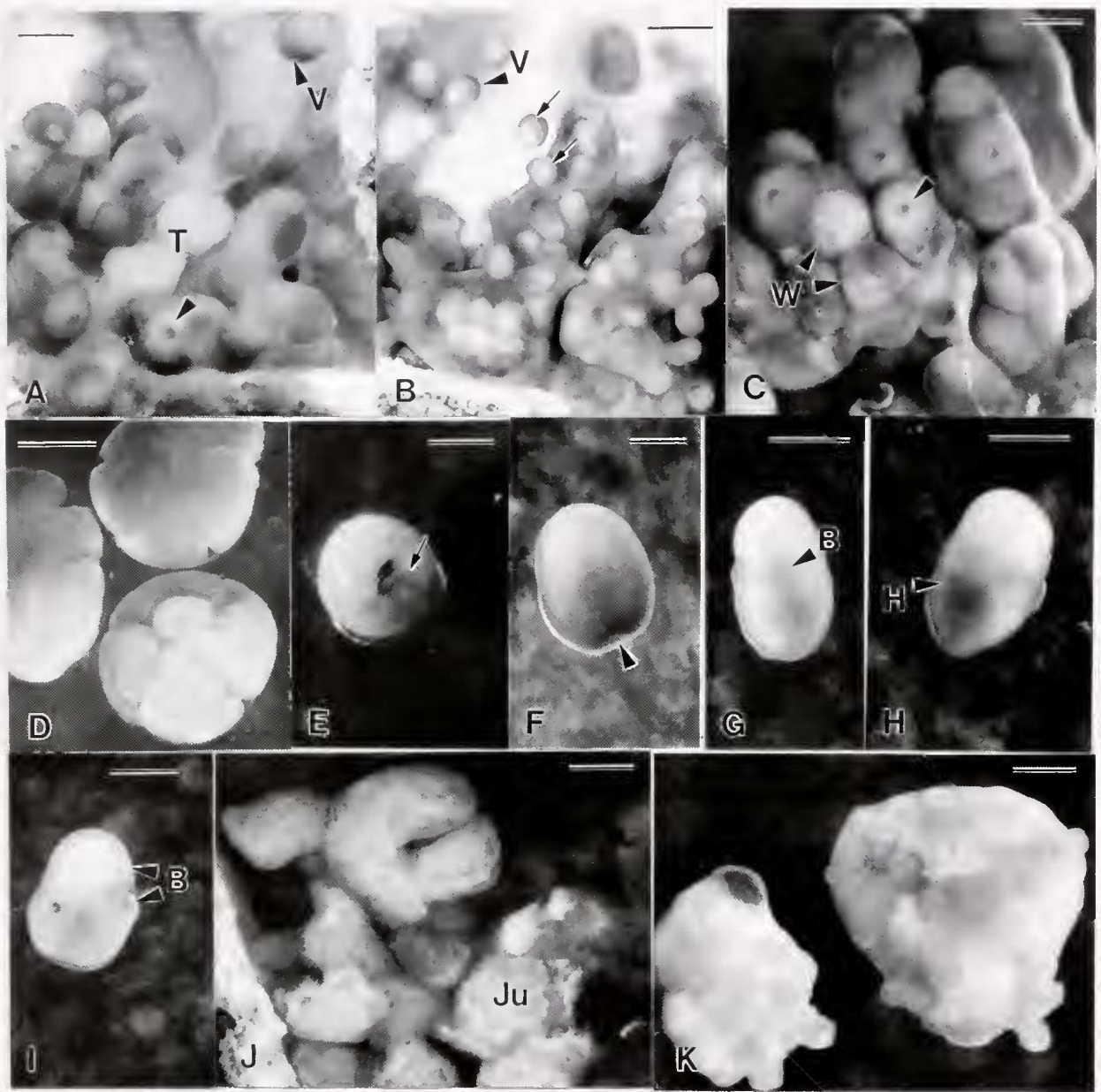
Figure 1. Light microscopy of *Cryptasterina hystera* gonads and embryos. **(A–C)** Ovotestes: The eggs have a dark vegetal region (V), and a dark spot marks the location of the germinal vesicle at the animal pole (arrowheads). The eggs float up from the dissected gonad (arrows). The gonad lobes are dominated by oocytes with occasional lobes filled with sperm (T). W, wrinkled blastulae. **(D)** Wrinkled blastulae. **(E)** Unhatched gastrula with black pigmented cells (arrow) streaming into the blastopore from one side of the embryo. The blastopore has a dark rim. **(F)** Hatched gastrula with dark pigment in vegetal hemisphere. Arrowhead, blastopore. **(G–I)** Brachiolaria larvae: The pigment remains in the posterior region. The lateral brachia (B) and hydroporic canal (H) are marked by darker cytoplasm **(J)** Gonad filled with near-term juveniles (Ju). **(K)** Moribund large juveniles dissected from gonad. Scales: A, C = 430 µm; B = 880 µm, D–F = 220 µm, G–I = 300 µm, J = 580 µm, K = 750 µm.

development in spermatocyte columns was typical of asteroid spermatogenesis. Spermatozoa were scattered in clumps intermingled with developing oocytes or in small pockets along the gonad wall (Figs. 2A, D; 3A). They remained separated from the oocytes by the follicle layer. The spermatozoa had a spherical head with the acrosome in a depression in the nucleus and surrounded by periacrosomal material, a midpiece, and a flagellum (Fig. 2F, G). These features are typical of asteroid sperm. When placed in seawater, the sperm of *C. hystera* exhibited typical motility.
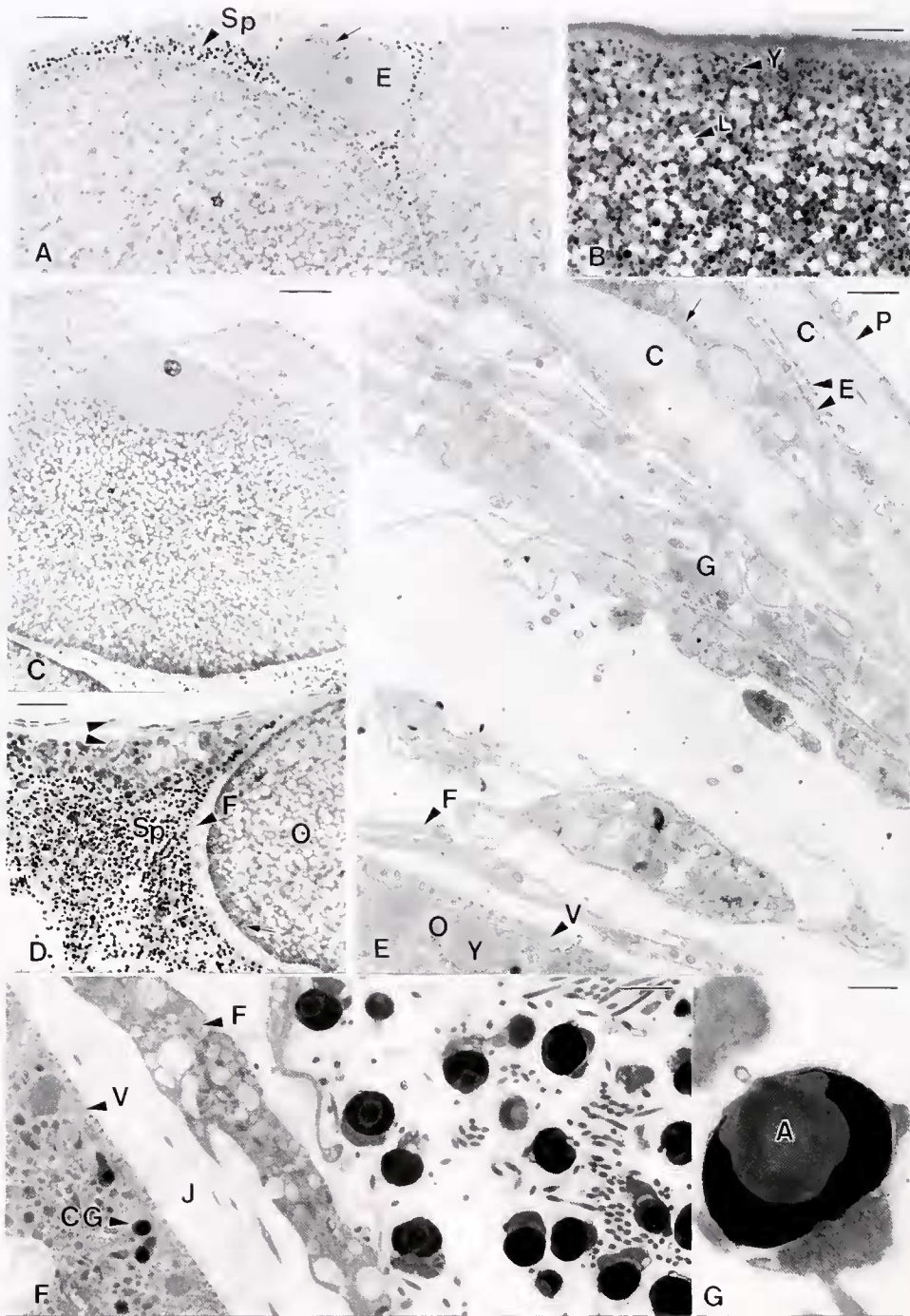
Figure 2. Light and transmission electron microscopy (TEM) of the ovotestis of *Cryptasterina hystera* (**A**) The early oocyte (E) contains a few yolk granules (arrow), and sperm (Sp) are present. (**B–C**) Advanced oocyte filled with lipid droplets (L). Yolk granules (Y) are dispersed among the lipid droplets and along the cortex. (**D**) Egg (O) and sperm (Sp) separated by the layer of follicle cells (F) around the egg. The arrowheads show the inner and outer tissue layers of the gonad wall. (**E, F**) TEM of gonad wall and oocyte (O). C, connective tissue layers; CG, cortical granule; E, epithelium of genital coelom; F, follicle cell; G, germinal epithelium; J, jelly coat; P, peritoneum; V, vitelline coat; Y, yolk granule; Arrow, phagocyte. (**G**) Sperm. A, acrosome. Scales: A, B, D = 50 μm; C = 100 μm; E = 1.5 μm; F = 2.0 μm, G = 0.4 μm.

Development

Embryos in all the gonads of individual *C. hystera* were usually at a similar stage of development, indicating that egg maturation, ovulation, and fertilization were synchronous (Figs. 1J; 3A, B, C). Among individual adults, however, the embryos were often at a different stage of development. Early in the season, cohorts of embryos developed alongside advanced gametes in the same gonad (Fig. 3A). By November and December, only a few unfertilized fully grown eggs remained in the gonad and were likely to be atretic. During these months, most gonads contained a clutch of juveniles (Fig. 1J)

Ovaries placed in 1-methyladenine (1-MA) released eggs, and many of these eggs were fertilized by sperm present in the gonad. Early cleavage was radial and holoblastic. The earliest embryos encountered in the gonad were wrinkled blastulae (Fig. 1C, D). Based on development in laboratory cultures, these embryos were about 12 h post-fertilization. Wrinkled blastulae had a highly contorted epithelium composed of cuboidal epithelial cells filled with large lipid droplets (Fig. 1C, D; 3C). The blastulae were enclosed in the fertilization envelope. The dark cytoplasm remained in the vegetal region throughout early development. In blastulae, it was confined to the lower third of the embryo. On removal from the gonad, blastulae floated at the air-water interface and continued development through the brachiolaria larval stage, metamorphosing into juveniles in 3 weeks.

The blastopore developed at the middle of the dark cytoplasm, about 24 h post-fertilization, and its rim was marked by dark pigment (Fig. 1E). During gastrulation, the dark cells around the blastopore did not move symmetrically into the embryo from around the margin of the opening (Fig. 1E). One side of the embryo appeared to contribute more dark cells to the developing archenteron than the other. As the larva elongated, dark pigmented cells moved anteriorly along the ventral surface and, to a lesser extent, the dorsal surface (Fig. 1F–H). The pigment did not extend beyond the midventral region. Although the distribution of the dark cytoplasm was variable among larvae, cells inheriting the dark pigment always remained in the posterior region of the larvae. In many larvae there was a contrast between the light anterior preoral lobe and the dark posterior region at the level of the brachiolar apparatus (Fig. 1I). The central brachium was light green, while the adjacent posterior body region was dark olive. On the dorsal side, dark cytoplasm marked the hydroporic canal (Fig. 1I).

The embryos hatched as late gastrulae at 40 h and the blastopore closed (Figs. 1F; 3A). Gastrulae and larvae swam propelled by their uniform cover of cilia. Their locomotion in gonad fluid depended on the availability of space. During the gastrula stage, lipid droplets were extruded into the blastocoel (Figs. 3D; 4A–C). Prior to extrusion, the epithelial cells changed from cuboidal to columnar, and lipid droplets were shunted to a basal location below the nucleus (Figs. 3D; 4A, B). This basal shunting was a prelude for lipid release. Lipid droplets were released into the blastocoel by an apocrine mechanism, taking part of the cell membrane with them (Figs. 3E; 4B, C). The lipid remained in the blastocoel throughout development (Figs. 3E; 4D).

Formation of the brachiolar attachment complex was first seen on external view in 5-day-old larvae as three protrusions at the anterior end (Fig. 1G). These were the developing brachia. By this stage the larvae swam at the bottom of the dish anterior end up, buoyed by the abundant lipid reserves in the blastocoel of this region of the larva. As the brachiolaria developed, the attachment complex became a prominent feature of the larva. The adhesive disc developed at the base of the brachia (Fig. 4D). Both the adhesive disc and brachiolar arms contained batteries of secretory cells that were presumably the source of adhesive material. Advanced larvae readily adhered to the substratum using their brachia and, prior to metamorphosis, attached to the surface of the culture dishes with the adhesive disc. The juvenile rudiment developed in the posterior region (Fig. 1H, I). Newly metamorphosed juveniles (600 μm in diameter, two pairs of tube feet per radius) from laboratory cultures had an amber hue due to their abundant lipid reserves. These reserves were mobilized during the perimetamorphic period. In sections of juveniles with a newly differentiated gut and well-developed skeleton, the remaining lipid droplets were seen scattered in the body wall (Fig. 3G).

Juveniles brooded by *C. hystera* appear to leave the gonad in a synchronous manner, as indicated by the appearance of hundreds of juveniles (800 μm diameter) in aquaria over a few hours. These juveniles had two pairs of podia in each radius. Some juveniles (1–2 per gonad) remained in the gonads for a longer time (Figs. 1K; 3F). These juveniles were large (1–4 mm diameter) and had four to six pairs of tube feet per radius. How they attained this size in the gonads is not known, but the presence of adjacent moribund juveniles indicated that they cannibalize their siblings. They may also utilize eggs as a source of food. The significance of this phenomenon is not clear because the presence of degenerating juveniles in the gonads indicates that at least some of them were unable to emerge from the gonad (Fig. 1K)

Discussion

Internal fertilization, intragonadal development, and live birth of juveniles is a rare form of propagation in marine invertebrates. Independent dispersive stages are completely deleted from the life history. In echinoderms, intragonadal incubation of embryos is known for one crinoid, four ophiuroids, four holothuroids, and five asteroids (Table 1). In addition, some dendrochirotid, psolid, and apodid sea
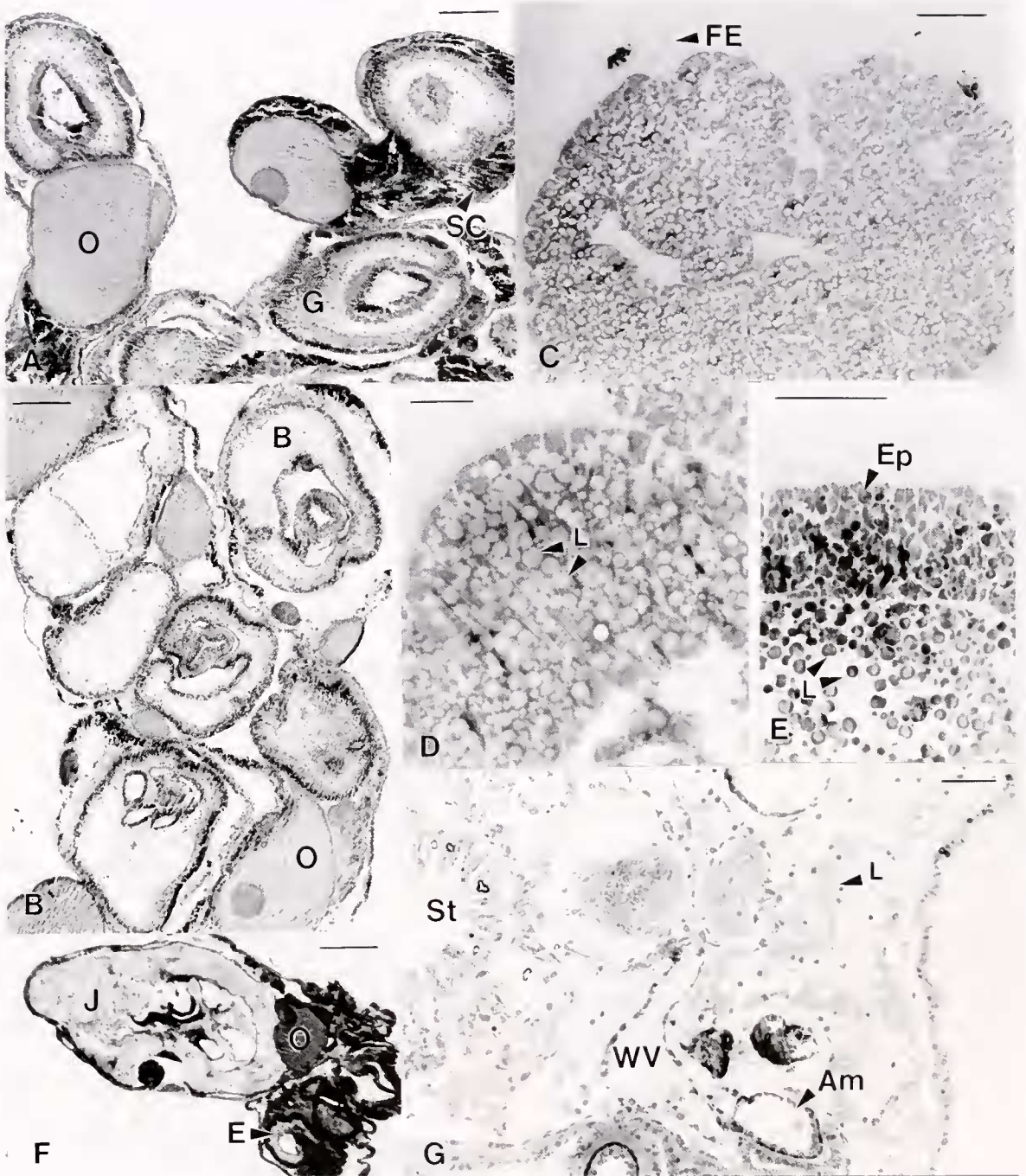
Figure 3. Light microscopy of *Cryptasterina hystera* gonads and embryos. **(A, B)** Gastrulae (G), brachiolaria larvae (B), oocytes (O), and spermatocyte columns (SC) in the gonads. **(C)** Wrinkled blastula with lipid droplets in cuboidal epithelial cells. FE, fertilization envelope. **(D)** Early gastrulae prior to extrusion of lipid (L). **(E)** Larva with lipid (L) in blastocoel. Few lipid reserves remaining in the epithelium (Ep). **(F)** Large juvenile (J), eggs (O), and embryo (E) in gonad. **(G)** Thin section of fully developed juvenile prior to the onset of feeding. The section extends from the stomach (St) to the edge of the body and shows lipid droplets (L) remaining in the body wall. WV, water vascular system; Am, ampulla of tube foot. Scales: A, B = 125 µm; C = 45 µm; D, E = 13.5 µm; F = 500 µm; G = 40 µm.
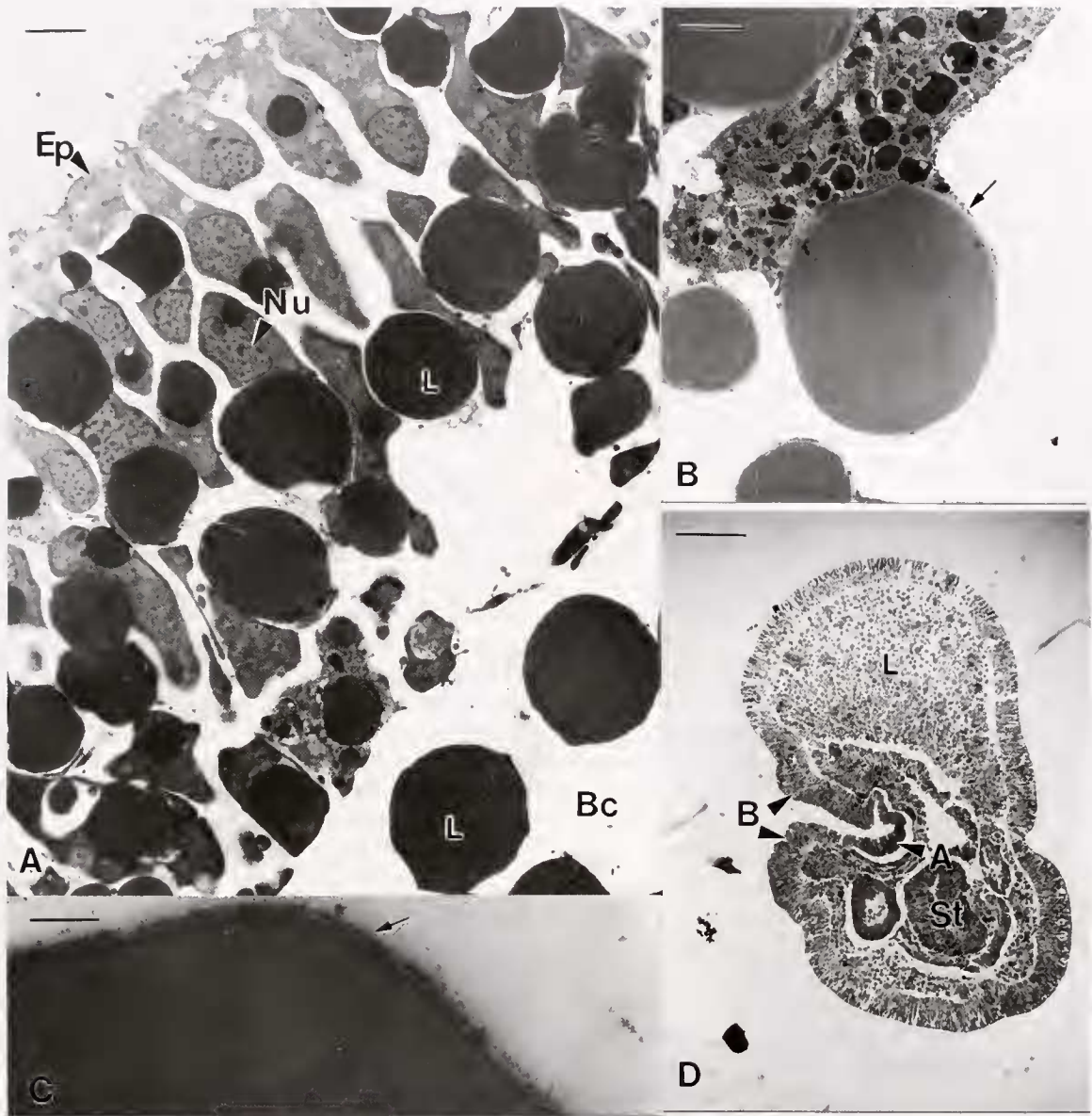
Figure 4. Light and transmission electron microscopy of *Cryptasterina hystera*. **(A–C)** Lipid reserves (L) are shunted basally below the nucleus (Nu) of the cells in the gastrula epithelium (Ep) before secretion into the blastocoel (Bc). The lipid is released by an exocrine mechanism, often taking a portion of the cell membrane (arrow). **(D)** Brachiolaria with lipid droplets (L) filling the blastocoel. A, adhesive disc; B, brachia; St, stomach. Scales: A = 3.0 μm; B = 1.5 μm; C = 0.15 μm; D = 85 μm.

cucumbers incubate their offspring in the coelom and juveniles emerge from the adult body (Reviews: Vaney, 1925; McEuen, 1986; O'Loughlin, 1994). Although live birth is rare in echinoderms, it appears that the potential for making the switch to this mode of propagation is more common in some taxa than others. The Holothuroidea contains the highest number of viviparous species (O'Loughlin, 1994). Aside from the aberrant *Xyloplax medusiformis*, all viviparous asteroids are in the Asterinidae (Table 1).

As is characteristic of viviparous asterinids, the gonads of

Cryptasterina hystera were ovotestes (Komatsu *et al.*, 1990; Byrne, 1996). This condition is also reported in viviparous holothuroids (Miller, 1985; Frick *et al.*, 1996). Despite their intragonadal location, the sperm of *C. hystera* had a well-developed tail, were fully motile, and could fertilize eggs *in vitro*. Their ultrastructure was the asteroid "echinosperm-type" (*sensu* Jamieson, 1985) with a round head and embedded acrosome. It is likely that these sperm would be functional if released into the sea. For outcrossing to occur in *C. hystera* and other viviparous echinoderms, sperm

Table 1

Incidence of intragonadal development in the Echinodermata

Class	Reference
Crinoidea	
Comatilia iridometriformis	Messing (1984)
Holothuroidea	
Leptosynapta clarki	Sewell and Chia (1994)
Oneirophanta mutabilis	Hansen (1968)
Taeniogyrus contortus	Vaney (1925)
Trachythyone mira	Ludwig and Heding (1935)
	O'Loughlin (2002)
Ophiuroidea	
Amphiura microplax	Mortensen (1936)
Amphiura monorima	Mortensen (1936)
Astrochlamys sol	Madsen (1967)
Ophionotus hexactis	Mortensen (1921)
Asteroidea	
Cryptasterina pacifica	Komatsu *et al.*, (1990)
Cryptasterina hystera	Byrne *et al.* (2003a)
Patiriella vivipara	Byrne and Cerra (1996)
Patiriella parvivipara	Byrne and Cerra (1996)
Xyloplax medusiformis	Rowe *et al.* (1988)

would have to gain access to eggs by swimming through the gonopore, an activity that would require sperm chemotaxis. Sperm chemoreception and sperm-activating pheromones have been documented for several echinoderms, including asteroids (Miller, 1989; Miller and Vogt, 1996). Asterinids with benthic development gather to lay communal egg masses, with sperm deposited directly onto the eggs (Tominaga *et al.*, 1994; Byrne, 1995). Sperm exchange in *C. hystera* would be facilitated by similar mating behavior. *C. hystera* clearly has the potential for self-fertilization. Determination of paternity for *C. hystera* and other viviparous echinoderms requires genetic investigation.

Developing oocytes of *C. hystera* remained within their follicle throughout development and were thereby separated from adjacent sperm. Asteroid follicle cells release 1-methyladenine (1-MA), which induces resumption of oocyte meiosis; the follicle cells themselves respond to the hormone by separating from the oocyte, freeing it for release (Schroeder *et al.*, 1979; Chiba, 2000). Oocyte maturation and ovulation occurred in *C. hystera*, but the spawning component of the 1-MA hormone activity cascade is suppressed (Mita, 1993). This fundamental change is a major alteration in reproduction and is associated with evolution of viviparity in this sea star. Spawning in sea stars involves contraction of the myoepithelial cells in the gonad wall. It is not known if these cells have been modified or are less abundant in the gonad of *C. hystera* than in the gonads of free-spawning asteroids. Once freed from the follicle, the eggs are no longer separated from endogenous sperm. In the laboratory, eggs released after 1-MA treatment were fertilized by sperm from the same gonad. It is not known if self-fertilization occurs in the field. In free-spawning echi-

noderms, sperm adhere to egg jelly coats that have been hydrated by seawater, and egg jelly induces the acrosome reaction (Sousa and Azevedo, 1985). It is not known if a similar change to the egg jelly and its role in acrosome stimulation occurs in the intragonadal environment.

From an ancestral state involving planktotrophic development and small eggs dominated by yolk protein (Byrne *et al.*, 1999b, 2003b), the increase in egg size in *C. hystera* was associated with a shift to lipid-dominated eggs. An increase in lipid reserves is characteristic of echinoderms with lecithotrophic development (Emlet *et al.*, 1987; Jaeckle, 1995). The initial phase of oogenesis during which yolk protein is sequestered into yolk granules is typical of the ancestral-type oogenic pattern (Byrne *et al.*, 1999c, 2003b). This is followed by hypertrophic lipid deposition, similar to that seen in the lipid-rich eggs of the sea urchin *Heliocidaris erythrogramma* (Byrne *et al.*, 1999c). Possession of a buoyant egg in *C. hystera* and its congener *C. pacifica* was not expected in species with intragonadal development. Development in *C. hystera* and *C. pacifica* is completely supported by maternal provisioning in the egg. In contrast, *Patiriella vivipara* and *P. parvivipara* have small (120-μm diameter), secondarily reduced negatively buoyant eggs with minimal lipid stores (Byrne *et al.*, 1999b). Due to reduced egg provisioning, development in the viviparous *Patiriella* depends on sibling cannibalism (Byrne, 1996), a form of matrotrophy occasionally observed in *C. hystera* and also reported in viviparous holothuroids (Frick, 1998).

The structure of the gonad of *C. hystera*, with the inner and outer tissue layers separated by the genital coelom, is typical of asteroids (Chia and Koss, 1995). With the exception of the presence of both eggs and sperm, the structure of the gonads did not indicate any morphological specializations that might be associated with the evolution of viviparity. Similarly, the structure of the gonads of viviparous *Patiriella* and viviparous holothuroids is similar to those of broadcast spawners (Sewell and Chia, 1994; Byrne, 1996). In contrast, echinoderms that brood embryos in non-gonadal structures often have specializations for transferring nutrients to developing young (Walker and Lesser, 1989; McClary and Mladenov, 1990). *C. hystera* appears to have no anatomical impediments for acquisition of a marsupial function for the gonads. Considering this lack of morphological constraint, it is surprising that a viviparous life history is not more common in asteroids.

Most of the nutritive provisions loaded into the egg of *C. hystera* are reserved for the postlarval perimetamorphic stage. Early development is burdened by excess nutritive reserves not required for embryogenesis. Shunting of lipid into the basal cytoplasm of the epithelium, as seen in *C. hystera*, is common in lipid-rich echinoderm embryos (Patent, 1968; Henry *et al.*, 1991; Cerra and Byrne, 1995; Byrne and Cerra, 2000; Byrne *et al.*, 2003b). This is suggested to be a mechanism for partitioning excess reserves

away from the active apical region of the cell (Cerra and Byrne, 1995). In *C. hystera*, most lipid reserves are extruded into the extracellular blastocoelic space at the gastrula stage by an apocrine mechanism, similar to that documented for the sea urchin *Heliocidaris erythrogramma* (Henry *et al.*, 1991). The blastocoel thus functions as a storage space for nutrients to support juvenile development. Lipid extrusion is now reported for the embryos of two echinoderm species, both of which have highly buoyant, lipid-rich eggs. Such extrusion is the extreme outcome of the basal shunting process and may be associated with the presence of a particular class of lipid.

The presence of differently pigmented cytoplasm, as seen in the eggs of *C. hystera*, has not been reported for asteroids but is known for one echinoid, *Holopneustes purpurascens*. The animal and vegetal hemispheres of the eggs of *C. hystera* could be discerned by light and dark pigment, respectively. This is similar (but opposite) to the situation in the eggs of *H. purpurascens*, where the animal pole has dark yolk and the vegetal pole has light yolk (Morris, 1995). In that urchin, dark pigment is segregated into one blastomere at the 2-cell stage. In *C. hystera*, by contrast, the pigment remained in the vegetal region, with the major cellular segregation occurring at the third equatorial cleavage that separates cells into animal and vegetal fates. The dark cytoplasm remained in the lower third of the embryo, and the blastopore appeared where the dark pigment was most concentrated. During gastrulation, it appeared that one side of the embryo contributed more dark cells to the developing archenteron than the other. The mechanism of gastrulation in *C. hystera* may differ from the radially symmetrical pattern of involution traditionally attributed to echinoderms. Asymmetrical cell movements during gastrulation have been reported for the lecithotrophic embryos of the sea urchin *Heliocidaris erythrogramma* (Wray and Raff, 1994) and also occur in the lecithotrophic embryos of *Patiriella exigua* (Byrne and Cerra, unpubl.). More studies are required to determine whether gastrulation by asymmetrical invagination of cells from the right and left sides of the embryo is a feature of lecithotrophic development in echinoderms.

After closure of the blastopore, dark-pigmented cells that remained evident on the surface view migrated ventrally. This is consistent with migration of the blastopore region to the ventral side of planktotrophic larvae that have a functional anus. In many larvae, the dark cells formed a boundary between the preoral-postoral regions on the ventral surface, at the level of the brachiolar apparatus. The pigment may thus mark the anterior and posterior cellular domains that in feeding larvae would be bordered by the preoral and postoral ciliated bands. This is reminiscent of the oral and aboral territories marked by the ciliary band in sea urchin plutei and for which patterns of gene expression have been determined (Davidson *et al.*, 1998). On the dorsal

side, dark pigment was also present in the posterior region, and the hydropore was strongly marked by dark pigment. The animal-vegetal pigment in the eggs of *C. hystera* has potential for use in cell lineage studies.

The presence of a functional brachiolaria in the gonads of *C. hystera* and *C. pacifica* indicates that these sea stars have the potential to brood and broadcast their young—an unusual form of poecilogony that has been reported for the sea star *Pteraster militaris* (McClary and Mladenov, 1988). The highly restricted distribution of *C. hystera* indicates, however, that the brachiolaria are not used for dispersal in nature and that the progeny are likely to leave the parent as juveniles (Byrne *et al.*, 2003a). In aquaria, only juveniles were released. In general, the biogeography of asterinid sea stars correlates with the presence or absence of a dispersive larva; the exception is the benthic developer *Patiriella exigua*, which occurs around the southern hemisphere (Waters and Roy, 2004; Colgan *et al.*, 2005). Viviparous asterinids have the most restricted distribution known for the Asteroidea (Byrne *et al.*, 1999a, 2003a). Three of these occur in Australia, and their distributions range from 50 km (three locations, *C. hystera*) to 100 km of coastline (seven locations, *P. vivipara*). It would be interesting to know the distribution of *C. pacifica*, the fourth viviparous asterinid, in Japan. The mangrove habitats where *C. hystera* is found are vulnerable to anthropogenic change, highlighting the need to determine the distribution of this new viviparous species.

Acknowledgments

Literature Cited

Byrne, M. 1995. Changes in larval morphology in the evolution of benthic development by *Patiriella exigua* (Asteroidea: Asterinidae), a comparison with the larvae of *Patiriella* species with planktonic development. *Biol. Bull.* **188:** 293–305.

Byrne, M. 1996. Viviparity and intragonadal cannibalism in the diminutive asterinid sea stars *Patiriella vivipara* and *P. parvivipara*. *Mar. Biol.* **125:** 551–567.

Byrne, M., and A. Cerra. 1996. Evolution of intragonadal development in the diminutive asterinid sea stars *Patiriella vivipara* and *P. parvivipara* with an overview of development in the Asterinidae. *Biol. Bull.* **191:** 17–26.

Byrne, M., and A. Cerra. 2000. Lipid dynamics in the embryos of *Patiriella* species (Asteroidea) with divergent modes of development. *Dev. Growth Differ.* **42:** 79–86.

Byrne, M., A. Cerra, M. W. Hart, and M. J. Smith. 1999a. Life history diversity and molecular phylogeny in the Australian sea star genus *Patiriella*. Pp. 186–196 in *The Other 99%: The Conservation*

and Biodiversity of Invertebrates, W. Ponder and D. Lunney, eds. Transactions of the Royal Society of New South Wales, Sydney.

Byrne, M., A. Cerra, and J. T. Villinski. 1999b. Oogenic strategies in the evolution of development in *Patiriella* (Asteroidea). *Invertebr. Reprod. Dev.* **36:** 195–202.

Byrne, M., J. T. Villinski, E. Popodi, P. Cisternas, and R. R. Raff. 1999c. Maternal factors and the evolution of developmental mode: evolution of oogenesis in *Heliocidaris erythrogramma. Dev. Genes Evol.* **209:** 275–283.

Byrne M., M. W. Hart, A. Cerra, and P. Cisternas. 2003a. Reproduction and larval morphology of broadcasting and viviparous species in the *Cryptasterina* species complex. *Biol. Bull.* **205:** 285–294.

Byrne M., P. Cisternas, P. Selvakumaraswamy, J. T. Villinski, and R. R. Raff. 2003b. Evolution of maternal provisioning in ophiuroids, asteroids and echinoids. Pp. 171–175 in *Echinoderm Research 2001,* J.P. Feral and B. David, eds. Balkema, Lisse, The Netherlands.

Cerra, A., and M. Byrne. 1995. Cellular events of wrinkled blastula formation and the influence of the fertilization envelope on wrinkling in the seastar *Patiriella exigua Acta Zool.* **76:** 155–165.

Chia, F.-S., and R. Koss. 1995. Asteroidea. Pp. 169–245 in *Microscopic Anatomy of Invertebrates,* F.W. Harrison and F.-S. Chia, eds. Wiley-Liss, New York.

Chiba, K. 2000. Meiosis reinitiation in starfish oocytes. *Zool Sci.* **17:** 413–417.

Colgan, D. J., M. Byrne, E. Rickard, and L. R. Castro. 2005. Limited nucleotide divergence over large spatial scales in the asterinid sea star *Patiriella exigua. Mar. Biol.* **146:** 263–270.

Dartnall, A. J. 1969. A viviparous species of *Patiriella* (Asteroidea, Asterinidae) from Tasmania. *Proc. Linn. Soc. N.S.W.* **93:** 294–296.

Dartnall, A. J., M. Byrne, J. Collins, and M. W. Hart. 2003. A new viviparous species of asterinid (Echinodermata, Asteroidea, Asterinidae) and a new genus to accommodate the species of pan-tropical exiguoid sea stars. *Zootaxa* **359:** 1–14.

Davidson, E. H., R. A. Cameron, and A. Ransick. 1998. Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. *Development* **105:** 3269–3290.

Degnan, B. M., and M. F. Lavin. 1995. Highly repetitive DNA sequences provides evidence for a lack of gene flow between two morphological forms of *Herdmania momus* (Ascidiacea: Stolidobranchia). *Mar. Biol.* **124:** 293–299.

Emlet, R. B., and O. Hoegh-Guldberg. 1997. The effects of egg size on post-larval performance: experimental evidence from a sea urchin. *Evolution* **51:** 141–152.

Emlet, R. B., L. R. McEdward, and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. Pp. 55–136 in *Echinoderm Studies,* M.J. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Frick, J. E. 1996. Evidence of matrotrophy in the viviparous holothuroid echinoderm *Synaptula hydriformis. Invertebr. Biol.* **117:** 169–179.

Frick, J. E., E. E. Ruppert, and J. P. Wourms. 1996. Morphology of the ovotestis of *Synaptula hydriformis* (Holothuroidea, Apoda): an evolutionary model of oogenesis and the origin of egg polarity in echinoderms. *Invertebr. Biol.* **115:** 46–66.

Hansen, B. 1968. Brood-protection in a deep-sea holothurian, *Oneirophanta mutabilis* Théel. *Nature* **217:** 1062–1063.

Hart, M. W., M. Byrne, and M. J. Smith. 1997. Molecular phylogenetic analysis of life-history evolution in asterinid starfish. *Evolution* **51:** 1846–1859.

Hart, M. W., M. Byrne, and S. L. Johnson. 2003. Cryptic species and modes of development in *Patiriella pseudoexigua. J. Mar. Biol. Assoc. UK* **83:** 1109–1116.

Hart, M. W., S. L. Johnson, J. A. Addison, and M. Byrne. 2004. Strong character incongruence and character choice in phylogeny of sea stars of the Asterinidae. *Invertebr. Biol.* **123:** 343–356.

Henry, J. J., G. A. Wray, and R. R. Raff. 1991. Mechanism of an

alternate type of echinoderm blastula formation: the wrinkled blastula of the sea urchin *Heliocidaris erythrogramma. Dev. Growth Differ.* **33:** 317–328.

Huber, J. L., K. Burke da Silva, W. R. Bates, and B. J. Swalla. 2000. The evolution of anural larvae in molgulid ascidians. *Semin. Cell Dev. Biol.* **11:** 419–426.

Jaeckle, W. B. 1995. Variation in the size, energy content and biochemical composition of invertebrate eggs: correlates to mode of larval development. Pp. 79–122 in *Ecology of Marine Invertebrate Larvae,* L. McEdward, ed. CRC Press, Boca Raton, FL.

Jamieson, B. G. 1985. The spermatozoa of the Holothuroidea (Echinodermata): an ultrastructural review with data on two Australian species and phylogenetic discussion. *Zool. Scripta* **14:** 123–135.

Keough, M. J., and A. J. Dartnall. 1978. A new species of viviparous asterinid asteroid from Eyre Peninsula, South Australia. *Rec. S. Aust. Mus.* **17:** 407–416.

Knowlton, N. 1993. Sibling species in the sea. *Ann. Rev. Ecol. Syst.* **24:** 189–216.

Komatsu, M., Y. T. Kano, and C. Oguro. 1990. Development of a true ovoviviparous sea star, *Asterina pseudoexigua pacifica* Hayashi. *Biol. Bull.* **179:** 254–263.

Ludwig, H., and S. G. Heding. 1935. Die Holothurien der Deutschen Tiefsee-Expedition. 1, Fusslose und Dendrochirote Formen. *Wissenschaftliche Ergebnisse der Deutschen Expedition Tiefsee auf dem Dampher "Valdivita" 1898–9.* **24:** 121–244.

Madsen, F. J. 1967. Ophiuroidea. *B.A.N.Z. Antarctic Res. Exped. 1929–1931 Rep. Ser. B. (Zool. Bot.)* **9:** 123–145.

McClary, D. J., and P. V. Mladenov. 1988. Brood and broadcast: A novel mode of reproduction in the starfish *Pteraster militaris.* Pp. 163–168 in *Echinoderm Biology,* R.D. Burke, P.V. Mladenov, P. Lambert, and R.L. Parsley, eds. Balkema, Rotterdam.

McClary, D. J., and P. V. Mladenov. 1990. Brooding biology of the sea star *Pteraster militaris:* energetic and histological evidence for nutrient translocation to brooded juveniles. *J. Exp. Mar. Biol. Ecol.* **142:** 183–199.

McEuen, F. S. 1986. The reproductive biology and development of twelve species of holothuroids from the San Juan Islands, Washington. PhD. thesis, University of Alberta.

Messing, C. G. 1984. Brooding and paedomorphosis in the deep-water feather star *Comatilia iridometriformis* (Echinodermata: Crinoidea). *Mar. Biol.* **80:** 83–91.

Miller, J. E. 1985. Viviparity in a psolid holothurian from the tropical western Atlantic. Pp. 472 in *Proceedings of the Fifth International Echinoderm Conference.* B.F.Keegan and B.D.S. O'Connor, eds, Balkema, Rotterdam.

Miller, R. L. 1989. Evidence for the presence of sexual pheromones in free-spawning starfish. *J Exp. Mar. Biol. Ecol.* **130:** 205–222.

Miller, R. L., and R. Vogt. 1996. An n-terminal partial sequence of the 13kDa *Pycnopodia helianthoides* sperm chemoattractant "startrak" possesses sperm-attractant activity. *J. Exp. Biol.* **199:** 311–318.

Mita, M. 1993. 1-Methyladine production by ovarian follicle cells is responsible for spawning in the starfish *Asterina pectinifera. Invertebr. Reprod. Dev.* **24:** 237–242.

Morris, V. B. 1995. Apluteal development of the sea urchin *Holopneustes purpurescens* Agassiz (Echinodermata: Echinoidea: Euechinoidea). *Zool. J. Linn. Soc.* **114:** 349–364.

Mortensen, T. 1921. *Studies of the Development and Larval Forms of Echinoderms.* G.E.C. Gad, Copenhagen. 261 pp.

Mortensen, T. 1936. Echinoidea and Ophiuroidea. *Discov. Rep.* **12:** 199–348.

Ó Foighil, D., and M. J. Smith. 1995. Evolution of asexuality in the cosmopolitan marine clam *Lasaea. Evolution* **49:** 140–150.

O'Loughlin, P. M. 1994. Brood-protecting and fissiparous cucumariids (Echinodermata, Holothuroidea). Pp. 539–547 in *Echinoderms*

Through Time, B. David, A. Guille, J.P. Féral, and M. Roux, eds. Balkema, Rotterdam.

O'Loughlin, P. M. 2002. Report on selected species of Banzare and Anare Holothuroidea, with reviews of *Meseres* Ludwig and *Heterocucumis* Panning (Echinodermata). *Mem. Mus. Vic.* **59:** 297–325.

Patent, D. H. 1968. The general and reproductive biology of the basket star *Gorgonocephalus caryi* (Echinodermata, Ophiuroidea). Ph.D. thesis, University of California, Berkeley.

Pocklington, P., and M. S. Hutcheson. 1983. New record of viviparity for the dominant benthic invertebrate *Exogone hebes* (Polychaeta: Syllidae) from the Grand Banks of Newfoundland. *Mar. Ecol. Prog. Ser.* **11:** 239–244.

Reid, D. G. 1990. A cladistic phylogeny of the genus *Littorina* (Gastropoda): implications for evolution of reproductive strategies and for classification. *Hydrobiologia* **193:** 1–19.

Rowe, F. W. E., A. N. Baker, and H. E. S. Clark. 1988. The morphology, development and taxonomic status of *Xyloplax* Baker, Rowe and Clark (1986) (Echinodermata: Concentricycloidea), with the description of a new species. *Proc. R. Soc. Lond. B* **233:** 431–459.

Schroeder, P. C., J. H. Larsen, and A. E. Waldo. 1979. Oocyte-follicle relationships in a starfish. *Cell Tissue Res.* **203:** 249–256.

Sewell, M. A., and F.-S. Chia. 1994. Reproduction of the intraovarian brooding apodid *Leptosynapta clarki* (Echinodermata: Holothuroidea) in British Columbia. *Mar. Biol.* **121:** 285–300.

Sousa, M., and C. Azevedo. 1985. Acrosomal reaction and early events at fertilization in *Marthasterias glacialis* (Echinodermata: Asteroidea). *Gamete Res.* **11:** 157–167.

Tominaga, H., M. Komatsu, and C. Oguro. 1994. Aggregation for spawning in the breeding season of the sea-star, *Asterina minor* Hayashi. Pp. 369–373 in *Echinoderms Through Time,* B. David, A. Guille, J.P. Féral, and M. Roux, eds. Balkema, Rotterdam.

Vaney, C. 1925. L'incubation chez holothuries. *Travaux de la Station Zoologique Wimereux* **8:** 254–274.

Villinski, J. T., J. L. Villinski, M. Byrne, and R. R. Raff. 2002. Convergent maternal provisioning and life history evolution in echinoderms. *Evolution* **56:** 1764–1775.

Walker, C. W., and M. P. Lesser. 1989. Nutrient and development of brooded embryos in the brittlestar *Amphipholis squamata:* do endosymbiotic bacteria play a role? *Mar. Biol.* **103:** 519–530.

Waters, J. M., and M. S. Roy. 2004. Out of Africa: the slow train to Australasia. *Syst. Biol.* **53:** 18–24.

Wray, G. A., and R. R. Raff. 1991. Rapid evolution of gastrulation mechanism in a sea urchin with lecithotrophic larvae. *Evolution* **45:** 1741–1750.